Sweet Corn Phytoglycogen as a Protein Stabilizing Excipient
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Introduction: Preservation of protein biotherapeutics is a major manufacturing burden that is typically addressed with expensive cold-chain storage. Sugar-based compounds that can stabilize proteins against physical and chemical degradation are receiving considerable interest as excipients that can be added to protein formulations. However, many of these compounds are acquired through complex synthesis and purification processes. Here, we demonstrate that the glucose dendrimer phytoglycogen produced by sweet corn can be used as an excipient to stabilize proteins during lyophilization. In contrast to other sugar-based excipients, phytoglycogen (“PG”) can be produced cheaply, extracted from sweet corn using simple methods, and is biocompatible.

Methods: We extracted PG from different maize sources (i.e., “PG1”-“PG16”). Solubility of each PG was tested by adding 1x PBS to a final expected PG concentration of 20 mg/mL concentration. Optically clear solutions were considered soluble PG, whereas cloudy solutions were excluded. PG cytotoxicity was tested using an established NIH 3T3 fibroblast cell assay [1]. Protein preservation was measured by subjecting lysozyme (2.5, 1.0, 0.25 mg/mL), green fluorescent protein (GFP) (15, 8, 4 µM), or horse radish peroxidase (HRP) (200 µg/mL) to repeated cycles of freezing, lyophilization, and resuspension in the presence or absence of PG (1-20 mg/mL). Solutions were frozen at -80°C and lyophilized. Lysozyme activity was measured after 10 cycles of lyophilization with the EnzChek™ Lysozyme Assay Kit (Invitrogen, USA), according to manufacturer’s instructions. GFP fluorescence was measured after 10 cycles of lyophilization via fluorimetry (ex/em 485/510 nm) using a SpectraMax M3 plate reader. HRP activity was measured after 10 cycles of lyophilization via Tetramethylbenzidine (TMB, 4 mg/mL) and 50 µL of H₂O₂ solution. Protein structure was analyzed by FTIR spectroscopy. A1650 cm⁻¹ represents amide 1 band, and A1550 cm⁻¹ corresponds to amide 2 band. The ratio of the area under the amide 1/amide 2 curve was used to assess changes in protein structure.

Results: PG 1, 2, 5, 8, 9, 11, 13, 15, and 16 were soluble up to 20 mg/mL in neutral-buffered saline, a common biotherapeutic vehicle. PG 1, 2, 9, and 13 were not cytotoxic to NIH3T3 fibroblasts at all concentrations in vitro. Stocks of GFP retained more than 80% activity over 10 lyophilization cycles when 20 mg/mL PG 1, 2, 9, 13 was added as an excipient, compared to less than 10% activity when no excipient was added. For example, GFP plus PG13 maintained at least 88% activity, whereas in the absence of PG13, GFP activity dropped to 5% after 10 cycles of lyophilization (Figure 1). As the concentration of PG decreased, the GFP activity after 10 cycles of lyophilization also decreased, demonstrating that the excipient activity of PG is dose-dependent.

Lysozyme maintained more than 83% activity over 10 lyophilization cycles when 20 mg/mL PG 15 or 16 was included in the formulation, whereas a significant decrease in activity was observed when PG was absent. Notably, when PG15 was added as an excipient, lysozyme activity was preserved to 99%.

HRP maintained at least 79% activity over 3 lyophilization cycles when 20 mg/mL PG 2 was added, while a significant decrease in activity was observed in the absence of PG. In particular, when PG15 was added as an excipient, HRP activity was maintained up to 95%.

FTIR was used to assess the structure of protein after lyophilization in the presence and absence of PG excipient. Including PG15 as an excipient preserved up to 95.5% of protein structural integrity, whereas only 39.8% of protein structure was maintained when PG15 was not included.

Collectively, these data demonstrate that PG excipients can stabilize proteins during freezing and lyophilization by maintaining protein structure. Future work will seek to identify the stabilizing mechanism of PG.

Conclusions: In conclusion, our data demonstrate that corn-derived phytoglycogen can provide a simple, biocompatible, abundant, and economically-favorable excipient to stabilize protein biotherapeutics, thereby reducing the need for expensive and complicated cold-chain storage.

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